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PRINCIPAL INVESTIGATOR: Silva Krause, Ph.D.

CONTRACTING ORGANIZATION: Tufts College
Boston, MA 02111

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14. ABSTRACT: The mammary gland is comprised of stromal and epithelial cells that communicate with each other through the extracellular matrix (ECM). Disruption of communication between the epithelium and stroma can both induce and promote breast cancer. Crosstalk between the mammary epithelium and stroma is also crucial for the proper patterning and function of the normal mammary gland. It has been proposed that HA may induce malignant transformation in normal cells through interaction with its receptors. We therefore wanted to elucidate its function during normal mammary gland development. The expression of HA in the stroma increased at week 5 and peaked at week 7, the time of puberty coinciding with ductal growth. We observed a decrease with age when the mammary gland achieves mature virginal development (week 9 and 11). The peak of HA expression during the time of puberty led us to hypothesize that HA expression may be estrogen-mediated. Preliminary data suggest a role of estrogen as a mediator for HA expression but the analysis is still ongoing.					
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Table of Contents

Cover.....	
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Outcomes.....	5

Introduction

The mammary gland is comprised of stromal and epithelial cells that communicate with each other through the extracellular matrix (ECM). Disruption of communication between the epithelium and stroma can both induce and promote breast cancer (1, 2). Crosstalk between the mammary epithelium and stroma is also crucial for the proper patterning and function of the normal mammary gland.

A major component of the ECM is hyaluronan (HA). HA is a very large polysaccharide, consisting of 2,000 to 25,000 repeating disaccharides of glucuronic acid and N-acetylglucosamine. HA serves both structural and signaling functions (3). HA as a negatively charged polymer provides remarkable hydrodynamic characteristics due to its viscosity and its ability to retain water. It therefore plays an important role in tissue homeostasis and biomechanical integrity. HA-induced signaling occurs through receptor interactions, such as CD44 and RHAMM (receptor for HA-mediated motility). These interactions activate numerous downstream signals such as Rac and Ras that mediate the cell's response to motility and migration.

HA is overproduced by many types of tumors and, in some cases, HA levels are prognostic for malignant progression (4, 5, 6). Several studies have reported a relationship between hyaluronan content and cancer progression and invasiveness in several types of cancer including mammary gland tumorigenesis (7, 8). However, HA has not been previously studied in mammary gland development and it is important to elucidate its role during development to better understand its function during mammary gland carcinogenesis.

Task 1: To examine HA levels in mouse mammary tissue during postnatal development.

In order to explore the function of HA during mammary gland development we looked at HA expression in the mouse mammary gland at 3, 5, 7, 9, and 11 weeks of age. The expression of HA in the stroma increased at week 5 and peaked at week 7, the time of puberty coinciding with ductal growth (Figure 1). We observed a decrease with age when the mammary gland achieves mature virginal development (week 9 and 11). HA is expressed in the periductal stroma, the outer boundaries of some adipocytes, and in some epithelial cells, most prominently in the terminal end buds (Figure 1).

The peak of HA expression during the time of puberty led us to hypothesize that HA expression may be estrogen-mediated. In order to test this hypothesis, we compared HA expression in the postnatal mouse mammary gland of mice that were ovariectomized at 25 days of age, i.e. estrogen-depleted, versus mice that were ovariectomized at the same age but in addition, had an estrogen pump implanted that released a steady amount of estrogen per day (releasing 2.5 $\mu\text{g/kg/day}$ E_2). We had a third group of mice that underwent a sham operation without any treatment. The mice were killed ten days later and mammary glands were analyzed for HA expression, CD44 expression and HA synthetases levels.

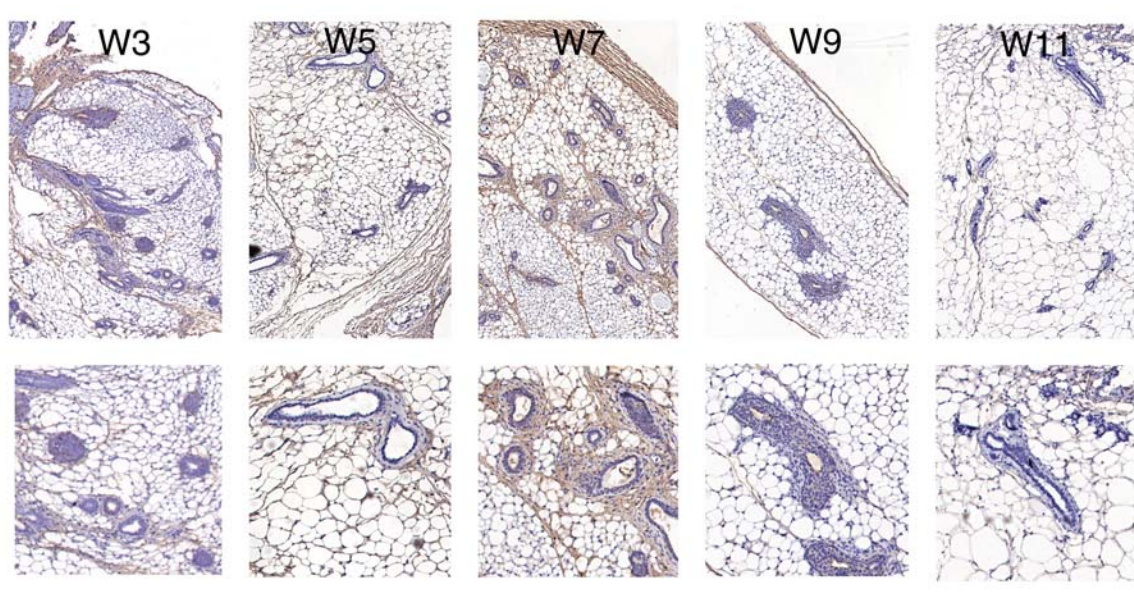


Figure 1: Expression of HA during postnatal mammary development.

HA expression in 3, 5, 7, 9 and 11-week old mice. Panels show histochemistry staining using biotinylated HA-binding protein (HABP). Peak expression of HA is observed at week 7. W stands for week. Lower panels show regions at higher magnification.

Preliminary data suggest a role of estrogen as a mediator for HA expression. Despite the fact that HA expression was not entirely depleted in the mammary glands of ovariectomized mice, there was no HA expression in the periductal stroma (area directly surrounding ductal epithelium) compared to a strong staining observed in control mice or mice that had an estrogen pump implanted (Figure 2). Further analysis is ongoing. For the expression of HA receptors, one of the isoforms of CD44, CD44v6, seems to have a different expression pattern in mammary glands of

estrogen-depleted mice but these observations have yet to be confirmed with repeat of protein analysis.

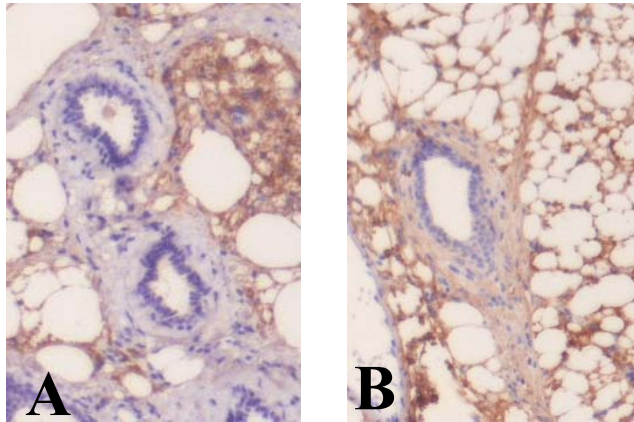


Figure 2: HA expression in periductal stroma.

Panels show histochemistry staining using biotinylated HA-binding protein (HABP) in mammary glands 35 days of age. Panel A shows lack of HA staining in periductal stroma of an ovariectomized mouse compared to a strong HA staining in panel B in the mammary gland of a mouse that had an estrogen pump implanted.

Task 2: To examine the effects of perturbing HA levels on mammary tissue morphology and polarization.

As we are still involved in the work for task 1 we have not begun to look at the role of HA in mammary gland carcinogenesis.

Key research accomplishments

- Hyaluronan showed a distinct expression pattern during mouse mammary gland development.
- An increased HA expression could be observed at week 5, peaked at week 7, the time of puberty, and decreased in week 9 and 11.
- Preliminary data suggest that one mediator for HA expression during mammary gland development is estrogen.

Outcomes

- Presented current data in a poster format at the DOD Era of Hope conference for breast cancer research in Philadelphia in June 2005.

Poster title:

“Involvement of extracellular matrix (ECM) components in mammary gland development.”

Conclusions

Preliminary data suggest a role of estrogen as a mediator for HA expression. Elucidating HA's role during normal mammary gland development will give important insights into possible functions during mammary gland carcinogenesis where HA is overexpressed.

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Training accomplishments:

July 2005: Attended workshop in Tissue Engineering at Tufts University, School of Bioengineering, Medford, MA.